Ab initio Study of the C12-C13 Conformation of 11-*cis*-retinal

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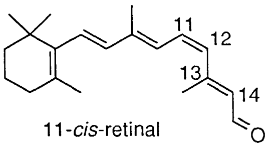
(Received August 3, 1995)

Ab initio calculation was performed to appreciate ^{13}C NMR chemical shift variations of 11-*cis*-retinal. The results show that isotropic shift of C12 varies significantly as the C12-C13 single bond rotates. This factor seems to be important to discuss a state of the chromophore in the protein.

The visual pigment rhodopsin possesses a 11-*cis*-retinal, which is covalently bound by a protonated Schiff base linkage with a lysine residue of opsin. The main absorpsion band of protonated Schiff base is 440 nm in methanol, whereas bovine rhodopsin absorbs at 498 nm.[1] Such a discrepancy is called "opsin shift," which is presumed to be caused by interactions between the chromopher and the apoprotein.



11-*cis*-retinal

The solid-state NMR measurements of 11-*cis*-retinal in bovine rhodopsin[2-4] revealed that the chemical shifts of C11 to C13 showed significant downfield shifts, compared to those of the protonated shiff base of 11-*cis*-retinal in $CDCl_3$. Such chemical shift differences reflect the protein-chromophore interaction, which probably contributes to the opsin shift. Han and Smith[5,6] gave an account for the shift difference by assuming that a carboxylate anion exists nearby C12, based on the point-charge model[7] and several mutagenetic studies.[8] They state that the chemical shift of C12 is almost independent of rotation of the C12-C13 single bond.[5] In their paper, a linear correlation is assumed between the chemical shifts and charge density. However, it is reported that if the π-conjugated system is broken by a torsion of the bond, chemical shifts are not in proportion to the charge density.[16] The present *ab initio* calculation shows significant angular dependence on the chemical shift. The magnitude of the variation is large enough to explain the difference of the chemical shift of 11-*cis*-retinal between in the protein and in solution.

We have used the program RPAC 9.0[9] which was developed by Hansen and Bouman for shielding calculations, interfacing to the GAUSSIAN90[10] program. Hartree-Fock MO calculations and geometry optimizations were carried out by GAUSSIAN92[11] using the basis sets of 4-31G.[12] All the calculations were carried out on Cray YMP8E / 8128 supercomputers. In the Hansen and Bouman theory,[13-15] the energy differences and matrix elements in the paramagnetic term are identified with the corresponding random phase approximation (RPA) transition energies and transition moments, and the localized orbital / local origin (LORG) scheme is used. The reliability of this method has been already confirmed by us through its application to *all-trans*-retinal and its related compounds.[16,17]

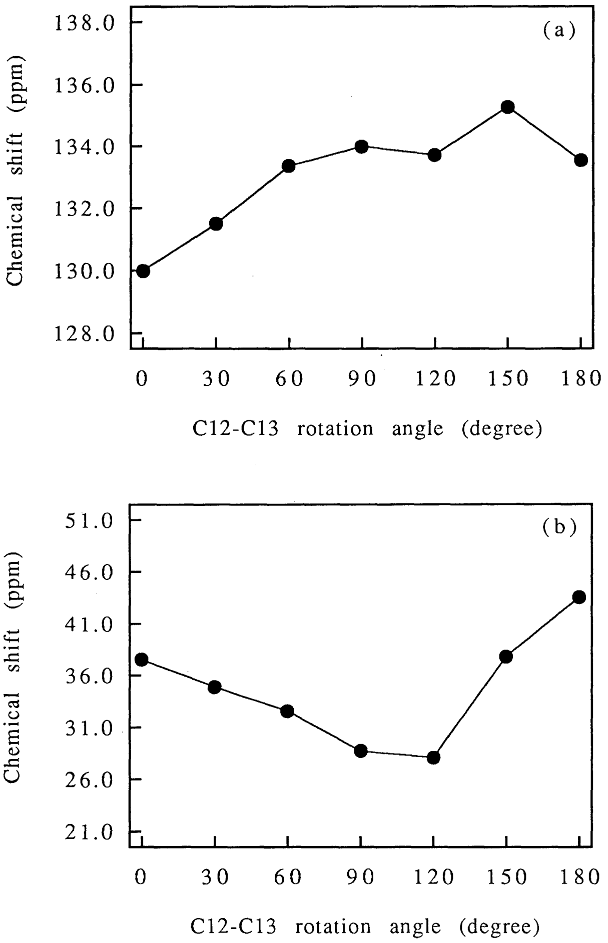


Figure 1. Calculated chemical shift of C12 of 11-*cis*-retinal shown in ppm from TMS. Isotropic shift (a), principal values of its shielding tensor: σ_{11} (b), σ_{22} (c), and σ_{33} (d).

Figures 1(a)-(d) show the calculated chemical shift values of C12 of 11-*cis*-retinal plotted against the rotation angle of C12-C13 single bond. The angle of 180° means planar *s-trans* conformation. Figure 1(a) is isotropic shift (σ_{iso}) and (b), (c), (d) are the principal values of shielding tensors: σ_{11}, σ_{22} and σ_{33}, respectively. The principal axis for σ_{11} is almost perpendicular to the conjugated planes. Those for σ_{22} and σ_{33} are in the plane, and the former is almost parallel to the double bond. The isotropic shift varies about 6 ppm depending on the rotation angle and shows a rather complex profile. On going from angle of 0° to 150°, σ_{iso} shifts downfield with a valley at 120°, and shifts a little upfield again at 180°. On the contrary, each component of the shielding tensor shows relatively simple dependence on the angle; σ_{11} has the most shielded value between 90° and 120°, σ_{33} has the

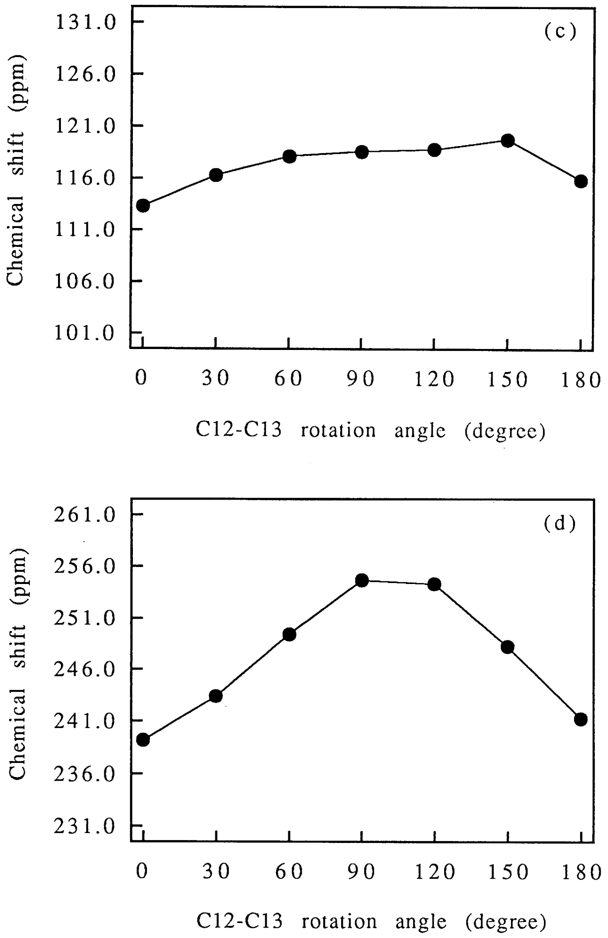


Figure 1. (continued)

least shielded value at similar point, and σ_{22} has less dependence on the rotation angle. The results show that σ_{11} and σ_{33}, which vary in opposite sense, dominantly contribute to the variation of σ_{iso}, causing its complex profile.

The calculated values do not need to reproduce the experimental values absolutely because they depends on the basis sets used in calculation. However, they would show the tendencies of variation of chemical shifts with the rotation. These results predict that the σ_{11} and σ_{33} would largely change with a dihedral angle of C11-C12-C13-C14 varying from 120° to 180°. As the angular dependence of these components are similar in magnitude and opposite in direction, if each component is affected by an external environment, isotropic shift would be change complicatedly. According to a conventional picture, σ_{11} reflects γ-steric effect. The σ_{11} value of C12 in bovine rhodopsin shows about 10 ppm upfield shift compared to that of the other retinals[2], but it cannot be explained only by the external negative charge model. As shown in Figure 1(b), σ_{11} occurs upfield shift in the range of 10 ppm when the C11-C12-C13-C14 conformation is distorted from planar *s-trans*.

As shown here, it is likely that the torsion of C12-C13 single bond contributes significantly to the shift difference of 11-*cis*-retinal in rhodopsin. It is thus necessary for one to consider the single bond torsion for discussing a state of the chromophore in the protein. We are now under investigation, revealing the correlation between molecular structure and experimental ^{13}C chemical shifts including the other carbons.

References and Notes

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